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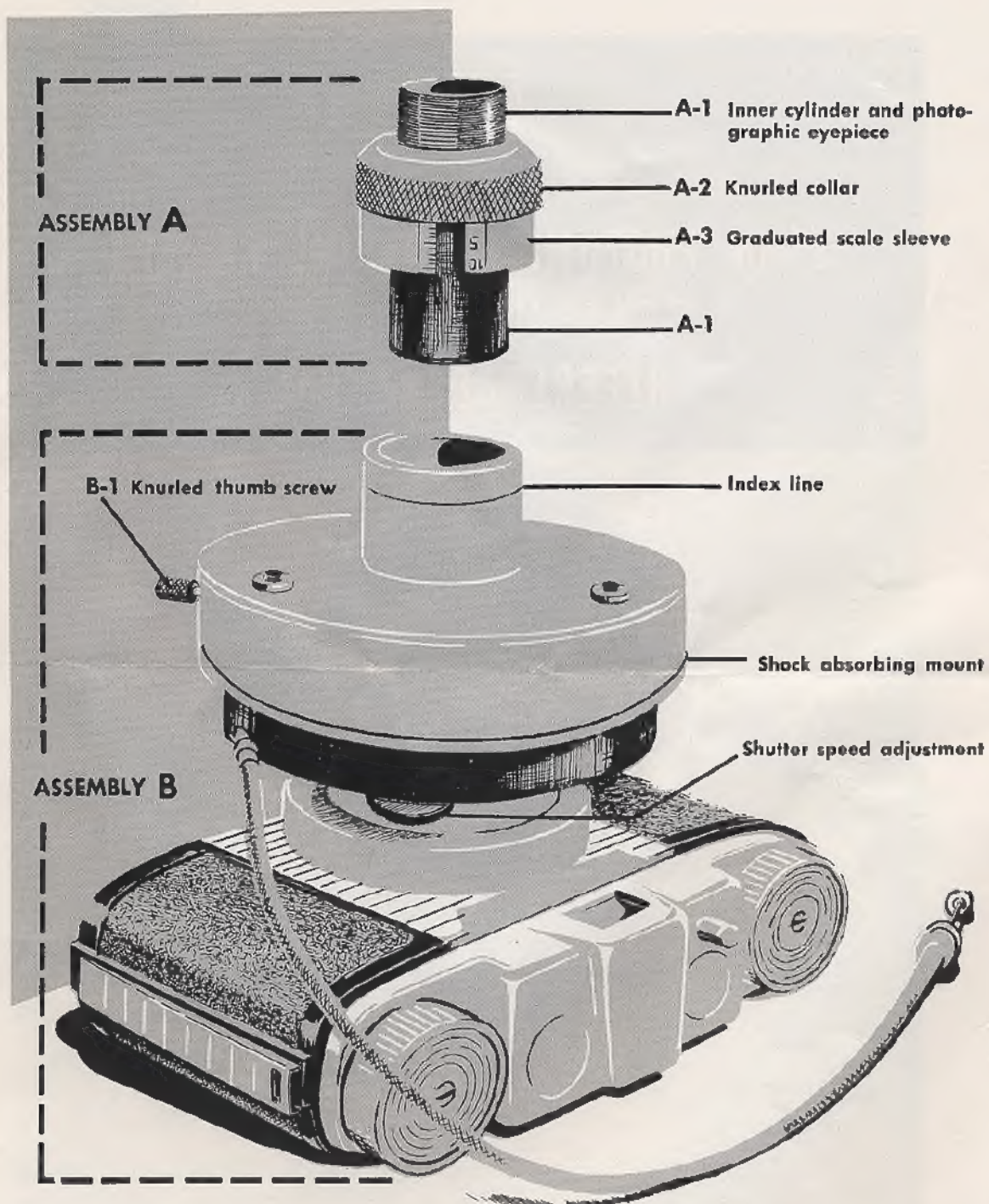
35MM PHOTOMICROGRAPHIC CAMERA

Model 635 For Trinocular Microstar

REFERENCE MANUAL



AMERICAN OPTICAL COMPANY • INSTRUMENT DIVISION • BUFFALO 15, N. Y.



AO Spencer 35mm Photomicrographic Camera
Model 635

AO SPENCER MODEL 635 PHOTOMICROGRAPHIC CAMERA 35MM

REFERENCE MANUAL

To effectively set up Model 635 Camera on MICROSTAR Trinocular Microscope, the user should carefully perform the following steps:

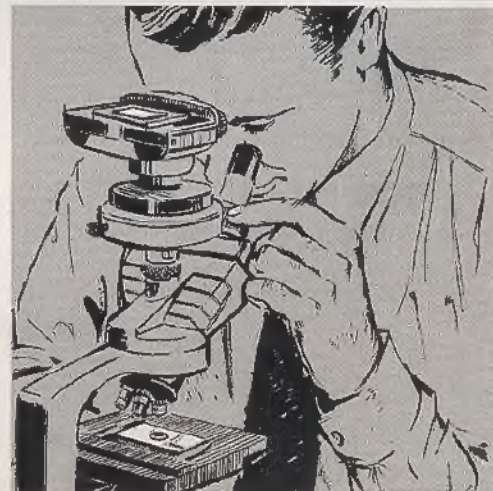
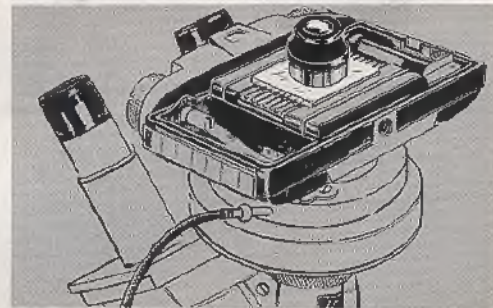
1. With camera back "down" loosen knurled thumb screw B-1.
2. Separate lens and graduated scale assembly A from the rest of the camera assembly B.
3. Rotate knurled collar A-2 of assembly A to expose approximately $3/8''$ of threads.
4. Unscrew vertical tube *only* from trinocular body by counterclockwise rotation.
5. Screw assembly A into trinocular body by rotating inner black cylinder A-1 to the full $3/8''$ excursion. Turn knurled collar A-2 clockwise until assembly is secure.

6. Insert assembly B into A and rotate assembly B until film winding knobs face the binocular tubes. Tighten knurled screw B-1.

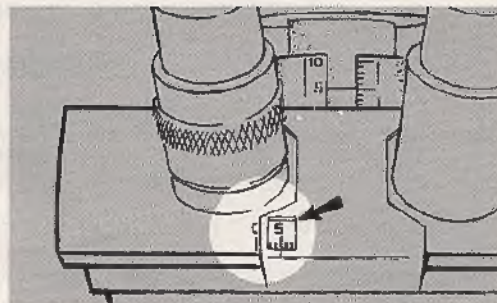
7. Remove camera back, set shutter at TIME and open it. Place ground glass (supplied with camera) over aperture, ground surface "down", and seat it on inner shoulders (film plane).

8. Place a stage micrometer or hemacytometer on the stage and with lowest magnification objective focus sharply through binocular system. Be sure to carefully adjust for eye refraction differences and interpupillary distance.

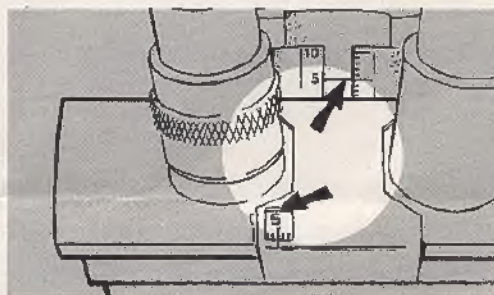
The final setting of fine adjustment and sharp focus is very important. To assure greatest possible accuracy bring the slide rulings into focus ten times (five upward and five downward). . . take reading of fine adjustment drum after each focus. . . average your readings. . . and set the fine adjustment to the average value.



9. DO NOT REFOCUS MICROSCOPE DURING STEPS 10 and 11. Note interpupillary value appearing in window below left eyetube.



10. Coordinate camera to visual system by placing a 10X or 15X photographic magnifier *directly* onto ground glass (a hand-held magnifier is inadequate) and focus the magnifier until cross-lines of the ground glass are sharply in focus. Loosen knurled collar A-2 slightly . . . retain hold of the collar and at the same time rotate entire assembly B until rulings are in sharp focus at plane of ground glass crosslines when viewed through photographic magnifier. Retain hold of assembly B and tighten knurled collar A-2 until secure.



11. Rotate graduated scale sleeve A-3 only, until index coincides with interpupillary value noted in step 9.

12. Close shutter and set at any position other than TIME . . . load camera and replace back . . . Camera and microscope are now ready for photomicrography by the user at any magnification.

OTHER USERS of the above camera outfit having different eye refraction and interpupillary distances should readjust the instrument by performing the following steps:

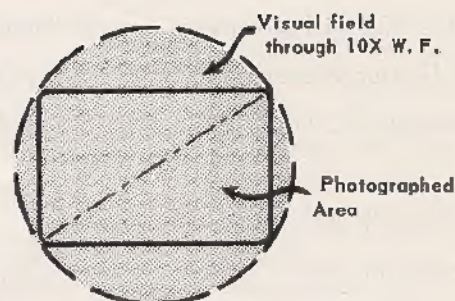
1. Focus microscope . . . adjust for eye refraction differences and interpupillary distance.
2. Note interpupillary value appearing in window below left eyetube.
3. Loosen knurled collar A-2 slightly. . . retain hold of collar and at the same time rotate assembly B until scale and index coincide with interpupillary value established in step 2.

SUPPLEMENTARY NOTES

Magnification and Field Coverage

The built-in photographic eyepiece contained in assembly A magnifies the objective image approximately $2\frac{1}{2}$ times. Consequently, resultant magnification at the film plane is the product

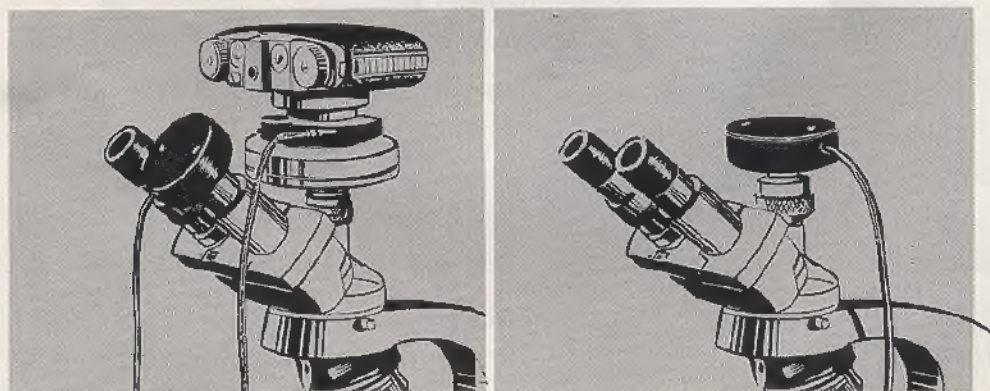
of the initial magnification of the objective times $2\frac{1}{2}$. The diagonal of the photographed field is equal to the diameter of the field viewed through 10X Wide Field eyepieces.



Filters and Exposure

Kodak Ektachrome Type F or AnscoColor Flash have the same Kelvin rating (3800K) and speed. Kodak light balancing filters 82C and/or 82A are required to raise the color temperature of the microscope illuminator up to the optimum 3800K. These filters are available in various sizes from the Special Products Sales Division, Eastman Kodak Company, 343 State Street, Rochester 4, New York.

Köhler type illumination is obtainable by focusing the condenser until the illuminator field diaphragm is in sharp focus at the specimen plane. Intensity of illumination should be modified by insertion of neutral density filters. . . not by reduction of voltage or closure of condenser aperture diaphragm. Swing-in auxiliary condenser (No. 231 clear glass) should be used only with 25 or 30 mm scanning objectives.



Exposure may be determined by removing the right eyepiece and inserting a photometer photocell into right eyetube. If a higher level of intensity is necessary, remove assembly B and position the photocell directly over assembly A. Approximately 15 times greater light intensity is registered over assembly A than in eyepiece tube.

Series 4 MICROSTAR is supplied with a built-in base illuminator and variable transformer. It is good practice to check the voltage at the secondary terminals. . . by means of a voltmeter . . . to make certain that the coil filament bulb is burning at approximately 7 volts. At this voltage,

EK filter 82C will produce good color when Kodachrome, Anscochrome or Ektachrome is used. The following exposures generally apply for average density slides when camera is loaded with Kodachrome. . . use 1/3 of speed indicated if faster Anscochrome or Ektachrome is used:

	<u>16mm</u>	<u>4mm</u>	<u>Oil immersion objective</u>
With EK 82C	1/10	1/5	1 second
Without filter	3/50	1/10	1/2

Photovolt readings taken through right eyetube of binocular body will read approximately: 9 units for 16mm objective; 4.5 units for 4mm; 1 unit for oil immersion if no filter is used. If EK 82C filter is used, approximately one-half of the above values are obtained (e.g.) 4.5; 2.25; 0.5 units.

Model 635 Camera Ibsor shutter mechanism is shock-mounted to prevent vibration difficulties. However, it is recommended that the cable release be used and that sufficient slack be allowed to prevent any unnecessary movement to the camera. The camera and shutter are light-tight. . . assembly B may be removed without danger of accidental film exposure.

Cleaning Procedure

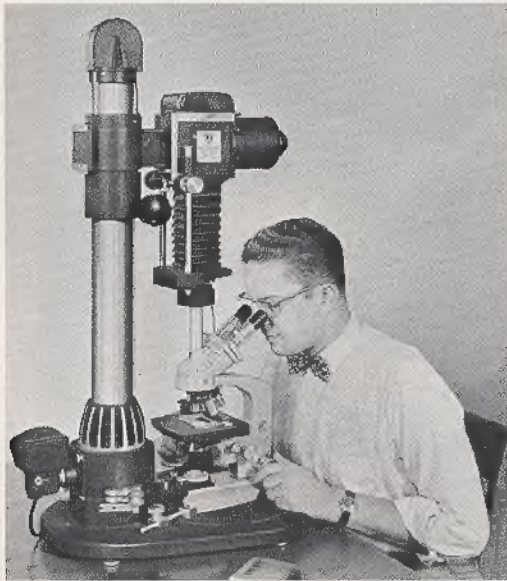
The exposed lens surfaces of assembly A should be cleaned carefully and only when necessary by the following steps:

1. Blow dust particles from surfaces with ear syringe.
2. Brush surface with a clean camel hair brush.
3. With the aid of a cotton swab (Q-tip) moisten the lens surface with weak detergent solution.
4. Wipe carefully with a well laundered lint-free soft cloth or a cotton tuft.

REFERENCES

- Photography Through the Microscope. 1952. Eastman Kodak Co., Rochester 4, New York. 68 pp.
- Photomicrography. 14 Ed. 1944. Eastman Kodak Co., Rochester 4, New York. 174 pp. The most useful single volume.
- Shillaber, C. P. Photomicrography in Theory and Practice. John Wiley & Sons, New York. 1944. VIII & 733 pp.
- Richards, O. W. Photography in Scientific Research. Journal of the Biological Photographic Association. May, 1954.
- Wall, L. C. Filters for Kodak Type F Color Films in Photomicrography. Medical Radiography and Photography. Eastman Kodak Co., Rochester 4, New York. Vol. 32, Number 1, 1956.

OTHER AO SPENCER PHOTOMICROGRAPHIC EQUIPMENT

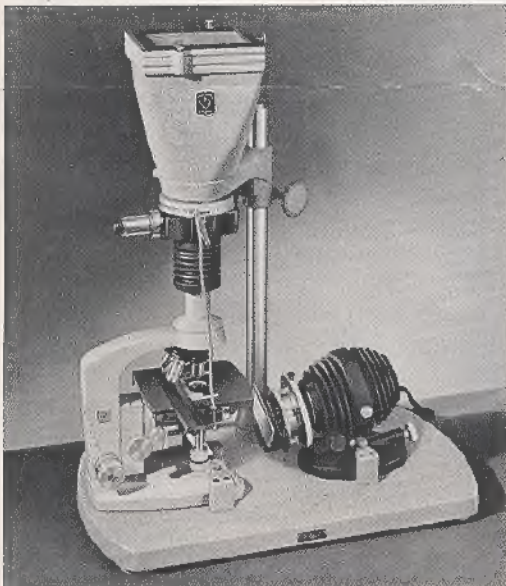


1300

MODEL 1300 ORTHOPHOT

Universal photomicrographic equipment adaptable for: photomicrography, photomacrography, general laboratory photography, cine-photomicrography and photocopying.

Informational brochure SB1300 is available on request.



682B

MODEL 682B

Photomicrographic Camera complete with base and arm, flexible light-tight adapter, focusing telescope with Universal shutter, 4" x 5" fixed camera back, two 4" x 5" Graphic double plate holders.

Alternate Backs For Model 682 Camera

No. 689 Polaroid Land Camera Back

No. 688 4" x 5" Graflok Camera Back

No. 668 35 mm Camera Back

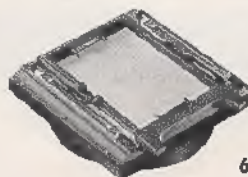
No. 669 Bantam Camera Back for E.K. 828 Roll Film

No. 672 Plate holder kit, 4" x 5" to 3 1/4" x 4 1/4"

Informational brochure "J" is available on request.



689



688



668



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EXPOSURE TABULATION

For No. 635 35mm Camera with Series 4 MICROSTAR



COLOR FILM

Kodachrome F; ASA-8

Kodachrome A and Ektachrome F; ASA-12

Anscoflash; ASA-16

Filter	3.5X, 5X, 10X Objectives			43X Objective			97X Objective		
	ASA-8	ASA-12	ASA-16	ASA-8	ASA-12	ASA-16	ASA-8	ASA-12	ASA-16
None	1/50	2/125	1/125	1/10	3/50	1/25	1/2	3/10	1/5
82A	1/25	1/50	2/125	1/5	1/10	1/10	1	3/5	1/2
82C	1/25	1/50	2/125	1/5	1/10	1/10	1	3/5	1/2
82A or 82C plus Didymium*	1/25	3/125	1/50	1/5	3/25	1/10	1-1/2	1	1
82A plus 82C plus Didymium*	3/50	1/25	1/50	3/10	1/5	3/25	2	1-1/2	1
Cobalt**	1/25	3/125	1/50	1/5	3/25	1/10	1-1/2	1	1
Cobalt** plus Didymium*	3/50	1/25	1/50	3/10	1/5	3/25	2	1-1/2	1

* Didymium 33mm filter 2.5mm thick (AO Cat. No. 608)
 ** Blue 33mm filter supplied with microscope (AO Cat. No. 402)

BLACK and WHITE FILM

Panatomic X and Microfile; ASA-25

Filter	3.5X, 5X, 10X Objectives	43X Objective	97X Objective
	ASA-25	ASA-25	ASA-25
None	1/25	1/25	1/5
58	1/50	1/10	1/2

MISCELLANEOUS DATA

Filter values:

- 82A = 1/2 stop
- 82C = 1/2 stop
- Cobalt = 1 stop
- Didymium = 1 stop
- #58 = 1 1/2 stop

635 35mm Camera has Ilex shutter with speeds T, B, 1/125, 1/50, 1/25, 1/10, 1/5, 1/2 and 1 second. To obtain additional exposure times as indicated in above tabulation proceed as follows:

- 3/10 (1/3 second) —set shutter at 1/10 and snap 3 times
- 3/25 (1/8 second) —set shutter at 1/25 and snap 3 times
- 3/5 (1/2 second) —set shutter at 1/5 and snap 3 times
- 3/50 (1/17 second) —set shutter at 1/50 and snap 3 times
- 3/125 (1/40 second) —set shutter at 1/125 and snap 3 times
- 2/125 (1/62 1/2 second) —set shutter at 1/125 and snap 2 times

NOTE: For best results check microscope and illuminator for following:

1. Transformer (# 350) should be set at highest voltage position.
2. Condenser should be properly focused and lamp iris set correctly.
3. Abbe Condenser iris should be correctly positioned.



DIGESTED HELPFUL HINTS ON 35 MM. PHOTOMICROGRAPHY - ESPECIALLY
COMPILED FOR GUIDANCE ON USE OF
THE AO SPENCER PATHOSTAR AND #635 CAMERA
Revised April 1957

A What contributes to faithful color reproduction?

- (1) Developing of film
- (2) Color temperature of bulb
- (3) Compensating filter(s)
- (4) Focus of condenser
- (5) Type of stain and contrast
- (6) Type of optics
- (7) Type of film used
- (8) Rigid adherence to prescribed technique of microscopy
- (9) Cleanliness of optics

B What variables affect exposure time?

- (1) Intensity of light source
- (2) Magnification
- (3) Speed of film selected
- (4) Focus of condenser
- (5) Cameras with bellows extension

C Can you minimize field curvature & how?

- (1) All microscopic images are curved or saucered
- (2) Reduce aperture of condenser
- (3) Focus off dead center
- (4) Project image greater distance

D How can magnification of film be established?

- (1) Project micrometer slide or haemocytometer ruling on ground glass placed in film aperture. Compare to millimeter rule, two known factors give you the unknown.

E Are apochromat objectives essential to good photomicrographs?

F Can the average user compete with professionals?

G Is meticulous microscope technique more essential in photomicrography?

H Which color film is preferred?

I Is black & white photomicrography relatively simple as compared to color?

J Reference data: exposures, film speeds, etc.

A-1 It is usually wise to send films for development to laboratory best equipped to serve your needs, both as to time and constancy of development. After some experimentation you can strike an exposure balance and technique that is compatible to a specific laboratory's development. It is obvious, therefore, that it is advantageous to channel your films to such a reliable facility.

- A-2 The rated color temperature of various bulbs is directly dependent upon applied voltage. If line voltage is lower than bulb rating, the light appears yellow; and conversely if line voltage is higher, the light is whiter. Raising voltage with variacs or transformers reduces life of bulb so the usual choice is compensating filters - see A-3. A simple 0-10 voltmeter (Triplex - trade name), obtainable from electronic supply houses, can be easily attached to transformer. This will permit exact match points for repetitive exposures and enables user to offset line fluctuations.
- A-3 Film manufacturers suggest corrective filters to raise color temperature to 3800 Kelvin, which is the correct temperature for Ektachrome, Kodak and Anscochrome. The extent of correction is a personal choice. Some prefer 82C alone; others in combination with 82A. Still others use the small blue glass furnished with microscopes. Trial alone will convince you the filter of choice. See table IV for filter factors.
- A-4 Substage condensers, and Abbe types in particular, often detract from color fidelity of photos. If condenser is focused correctly, very little color is introduced. If focused too high, blue will predominate; and if too low, red is in evidence. We cannot too strongly urge you to meticulously adhere to published procedures. On the AO Spencer #4 the field iris should be sharply focused in the image plane. Use a 16mm. objective and focus a slide, then adjust condenser until the closed lamp iris leaves are sharply focused. This setting is then ideal for all power objectives as well as for all slides of same relative thickness. To determine relative setting of condenser iris, remove ocular and close down until the leaves are restricting the light in the back lens of the objective. This materially changes light transmission.
- A-5 The color of stained microscope specimens contributes materially to the finished photo. Usually lacking in contrast there is some temptation to experiment so as to improve results. See table I.
- A-6 Apochromats, and coated achromats to a lesser extent, provide for slightly better color fidelity. See E.
- A-7 There is a choice of color films available. Authorities claim advantages and disadvantages for each type, so far as color reproduction is concerned. Trial is the best method to determine the one best suited to individual preferences.
- A-8 Diaphragm settings, both condenser and light source, can increase or decrease contrast and color. Carefully adjusted microscope assures optimum results.
- A-9 Even slight films on objective, especially the 4 mm. objective, will reduce resolution and color balance. Here too you can help assure optimum results - a good point to constantly check.

- B-1 The light intensity is of prime importance so far as exposure factors are concerned. Line voltage, bulb wattage and condenser iris settings all combine to provide variables. A sensitive exposure meter can accurately measure significant differences. Without same you can predict some errors - same as in conventional photography. Thickness of specimen has little or no influence on exposures. Where photometers are used, readings for references match should always be made with objective focused. Then move slide to clear area for uninterrupted light. Take reading with Photovolt 200M or similarly sensitive meter. If a reading of 30 is taken for a 16 mm. objective, and test roll indicates 1/50 is correct exposure, this can then be used to judge exposures for high dry or oil. For instance, a reading of 15 would then indicate 1/25, and of 5 would be approximately 1/10 second. Good, consistently uniform transparencies can be taken without photometers, provided all variables are normalized. A few rolls of film will indicate need of photometer.
- B-2 As magnification is increased, so is exposure time and in somewhat of a straightforward relationship. The suggested exposure table I more or less confirms the statement. Some prefer to use the numerical aperture of objectives as to comparative criteria, i.e. 16 mm. .25 N.A. about 1/5th the exposure of an immersed 97X 1.25 N.A. So much depends upon condenser iris setting and focus that you might prefer to ignore this as a yardstick.
- B-3 Only to serve as a reminder because it is so obvious, the film speeds are important factors of consideration.
- B-4 An incorrectly focused condenser can materially affect light transmission and exposure.
- B-5 Cameras provided with bellows permit longer projection. Light intensity varies inversely with the square of the distance. Simple mathematics will therefore permit computation where changes of this nature are involved.
- C-1 Objectives are notoriously curved or saucered. Paradoxically apos more so than achromats because of high apertures. All makes share this disadvantage and, except in rare instances, it is impossible to differentiate. Oculars can, with diaphragms, minimize, but for a given field coverage they are surprisingly uniform.
- C-2 Closing condenser iris will reduce curvature, but only at sacrifice of aperture and resolving power.
- C-3 The best way to reduce curvature is to focus off dead center. Try to spot an area about 1/3 the distance from center to the periphery and establish critical image at that point - it helps.
- C-4 With the most elaborate photo cameras you can project a greater distance to the film and only register the central, in-focus area. Although desirable, this radically reduces the area of tissue or preparation that is photographed.

- D-1 Project micrometer slide or haemocytometer ruling on ground glass placed in film aperture. Compare to millimeter rule, two known factors give you the unknown.
- E Apochromatic objective with corrected oculars and condensers will permit, but definitely do not assure, improved photos. More critically constructed and designed, they demand flawless technique to yield their inherent advantages. Some contend they are too time-consuming whereas others accept the bitter with the sweet. Like many points of issue, it is simply a matter of personal experience and preference.
- F If you demand speed and convenience and, to a limited extent, price, it is impossible to compete with both experience and more complicated and costly equipment. Many color slides projected at meetings are taken by expert photographers. The time involved, even by such a proficient technician, might be far more than you would care to devote. Frequently several exposures with various filters are shot, just to achieve the ultimate. Moreover, many slides, routinely cut and stained, cannot be compared to those specially selected and stained; the types usually involved in research projects, etc.
- G The degree of perfection achieved is directly proportional to the technique followed. Visual imagery is quite unlike what the film records. There is no accommodation in photography. Such faults as to unclean optics, condenser and lamp irises opened or closed beyond the norm, or lack of adequately centered light - all show up as flagrant errors with film. Books are written on this subject and this is but a gentle reminder that the end result is dependent upon adherence to good microscopy. Generally speaking, fine detail can only be obtained with thin sections 4 to 5 microns. You should record detail on film to correspond to the visual image.
- I Black & white photomicrography allows somewhat more latitude but it is equally important to follow rigid rules to assure negative quality that will enlarge 5 to 10 times. The choice of appropriate filters (Wratten) is more complex than is the case with color film.
- J LAB INDEX DATA
Most bulbs burn at close to 3000 Kelvin, IF line voltage closely matches bulb voltage. Correction filters suggested below are recommended by manufacturers but you may prefer less blue, and use one instead of two. The 82A & 82C, series V, 33 mm. diameter, can be purchased in larger photo outlets or direct. Larger sizes preferred for setups involving separate microscope illuminators.

Table I

Following taken from reference manual

Type Film	Kelvin Rating	ASA	Kelvin Microscope Lamp	Filters	Corrected to
Kodachrome A	3800	12	3000 ? +	82A	3400
Kodachrome F	3800	8	3000 ? +	82A&C*	3800
Ektachrome F	3800	12	3000 ? +	82A&C*	3800
Anschochrome Flash	3800	16-20**	3000 ? +	82A&C	3800

* If less blue desired, use 82C only - increases speed 1/2 stop.

** Speed of 12 with 82A & C and 20 with 82C only.

As a start with the #4 and 635 camera, we suggest following exposures. Transformer set at #7, regular blue glass supplied with microscope, and iris settings to correct opening. If 82A & 82C available, use one or both for trial run.

H & E stain or any predominantly red can be enhanced with use of Didymium filter (our #608 33 mm. dia., 2-1/2 mm. thick). Can be used in conjunction with 78A or 78B, 82A or 82C, or even the 33 mm. plain blue filter regularly supplied with Pathostar. Increase exposure 1 full stop when using this filter. Here again, the degree of blue desired in color transparencies is a matter of personal preference. The Didymium will accentuate the reds to give better balance. Eastman Panatomic X (new type) now available in 21 or 36 exposure magazines, can be used for trial run. Exposure table III below.

You will probably find that 78A, 78B, 82A or 82C filters decrease speed of film by 1/2 stop each; Didymium by 1/2 stop; regular blue supplied with microscope - 1 stop. Suggest you take successive exposures, with and without filters, using chart as guide, and decide your preference, for one or more corrective filters. Judge color on projected transparency, because yellow light neutralizes blue tones.

Table II Trial Run Koda A & F No filters Trial Exposures

3.5x - 5X - 10X objectives	1/50	1/25	1/10
43X	1/25	1/10	1/5
Oil immersion	1/5	1/2	1 Sec.

Polarized light with crossed polarity 2 sec (10X) 5 sec (43X) 10 sec (97X)

A Photvolt #200M can be placed in right binocular eyepiece tube and readings noted. This will provide reference for future exposures.

Table III

	Film		Anscoc Flash 82C	82C + Dyd	Panatomic X + X1 Filter
	Koda A + F Ekta F W/82C	82C + Dyd			
3.5X-5X } 10X	1/25	3/50	1/50	1/25	1/10
43X	1/5	3/10	1/10	3/25 or 1/5	1/2
97X	1 sec	1-1/2	1/2	1 sec	2 sec

The above will be close to desired exposures for transformer set at #7 (7 volts) but personal preference might indicate slight changes. You can click shutter 3 times at 1/25 in order to expose 1/8 second or 3 times at 1/10th for 1/3 second exposure.

+ Wratten X1 well suited to use with black and white (D-76 or Microdol suggested by EKCo.) Reported grain free at 8 times enlargement.

Table IV

Approximate filter factors

78A, 78B, 82A, 82B	1/2 stop	
Blue substage glass supplied with microscope		1 stop
Dydimium 2-1/2 mm. thick	1/2 stop	our #608
5 mm.	1 stop	
Wratten X1	2 stops	(4X)